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An Automated Exposure System for Human Inhalation Study

YU-SHENG LIN

THOMAS J. SMITH

Department of Environmental Health

Harvard School of Public Health

Boston, Massachusetts

PENG-YAU WANG

Graduate Institute of Environmental Engineering

National Central University

Chun-Li, Taiwan, Republic of China

ABSTRACT. The authors developed a computer-based exposure system for human inhalation research to study the toxicokinetics of gases. The system uses a set of computer-controlled solenoid valves that regulates inhaled gases and collect exhaled breaths from the subject in accordance with a predetermined time schedule. The volunteer's breathing activity is monitored simultaneously with a real-time instrument, calibrated by both internal and external standards. The common air pollutant, 1,3-butadiene, was used for system validation. The results indicated that the errors were less than 3% relative to reference standards, and the error in the timing of the valve operation was negligible. The average difference between breath-monitoring methods was within 10%. The proposed system is an easy-to-use, reliable device for human studies; more than 130 subjects were tested in a companion study.

<Key words: automated, exposure, gas, human, inhalation, vapor>

FOR DECADES, INVESTIGATORS have used inhalation chamber techniques in animal studies to study the toxicokinetics of gases or vapors.^{1,2} However, extrapolation of animal data for the estimation of human risks, on the basis of weight or surface-area scaling, is of concern because the anatomic structures, physiological mechanisms, or metabolic pathways are most likely different among species. For example, there are significant interspecies differences between rodents and humans in the major metabolic pathways for 1,3-butadiene (1,3-BD), a common air pollutant.^{3,4} Although individuals have used human *in vitro* data to bridge the gap, there remains a high degree of uncertainty when scaling human tissue and microsomal findings on metabolism to whole-body estimates.^{5,6}

Application of inhalation chamber technologies for toxicokinetics studies in humans is limited because chambers are expensive to build, difficult to maintain, and require significant manpower to operate.⁷⁻¹⁰ These factors make it difficult for researchers to conduct a study with a large human population (typically, no more than 50 human volunteers have been tested). Given that there is a wide range of variation in physiological and genetic metabolic factors across individu-

als,¹¹ it would not be appropriate for us to use a group average value based on a small sample size of volunteers. In addition, there are considerable measurement errors with these techniques, such as losses of test gases via adsorption, leakage, and possible reactions.^{12,13} There is a need for an approach that can easily and inexpensively (1) conduct a large-scale human study, and (2) collect reproducible inhalation kinetic data.

In this study, our objective was to develop a simple and reliable computerized exposure system that could be used to test many human subjects exposed to several inhaled test gases. There were 3 design aims: (1) provision of accurate exposure levels of test gases, (2) attainment of precise timing control of exposure and breath sampling, and (3) accurate measurement of a subject's breathing pattern. The system presented herein was successful in obtaining 1,3-BD kinetics, including uptake and *in vivo* estimates of metabolism for 133 subjects.

Materials and Method

Overview of the inhalation-exposure system. The automated inhalation system (Fig. 1) has 4 major component parts: (1) supply of test gas and gas delivery, (2) lim-

ing control of the experiment, (3) respiratory monitoring, and (4) breath sample collection. With the automated inhalation system, a subject can inhale the test gas or clean air through 1-way valves in a breathing face mask. Solenoid valves control the type of gas inhaled, as well as the collection of breath samples; the operation of solenoid valves is controlled in accordance with a predetermined schedule set by a computer program. The subject's breathing activity is monitored with a real-time breathing monitor, which is calibrated by both internal and external standards before and after an inhalation experiment. 1,3-BD was used for the evaluation of this inhalation system because this chemical is a gas with negligible skin absorption, and it is one of the U.S. Environmental Protection Agency's (EPA's) priority pollutants. The exposure

apparatus is not patented, and there are no plans to seek a patent; the authors have no financial interest in the apparatus or in any of the component systems.

Supply of test gas and gas delivery. We prepared the test gas (2.0 ppm 1,3-BD) by injecting 200 μ l of pure 1,3-BD vapor (i.e., > 99.99%, inhibited with *tert*-butylcatechol) from a gas steel cylinder (Aldrich Chemical Co. [Milwaukee, Wisconsin]) into a 100-l Tedlar™ bag (BGI, Inc. [Waltham, Massachusetts]) that contained purified air. The air was charcoal-filtered and dried with a molecular sieve from a standard gas generator (Model 340-53-S, VICI Metronics, Inc. [Houston, Texas]). A 200-ml gas sample taken from the Tedlar™ bag was analyzed, thus assuring that the 1,3-BD concentration was controlled at 2.0 ± 0.1 ppm during daily preparation.

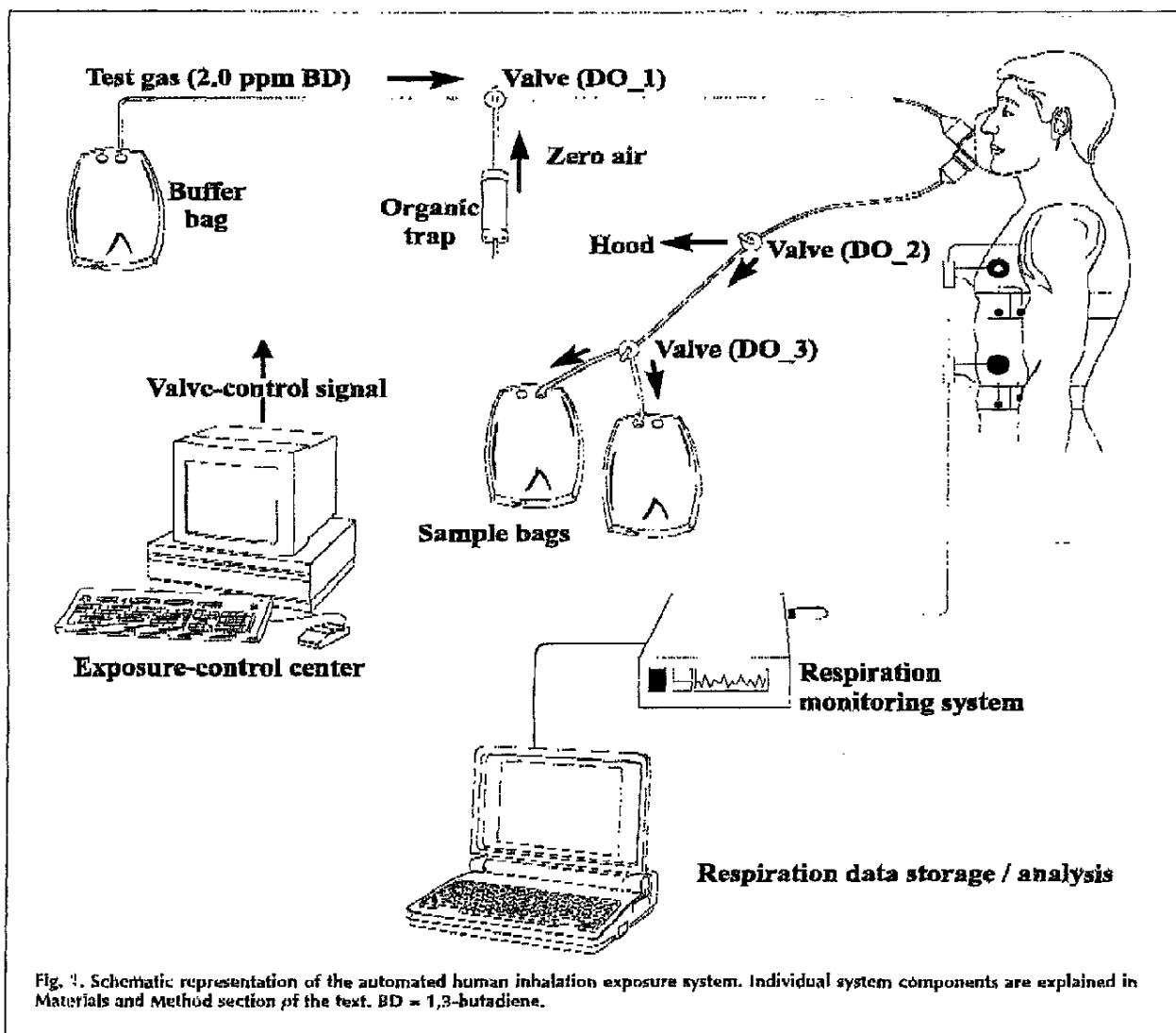


Fig. 4. Schematic representation of the automated human inhalation exposure system. Individual system components are explained in Materials and Method section of the text. BD = 1,3-butadiene.

We used 100-l Tedlar™ bags as the buffer bags (reservoir) of breathing gas for the human subjects.

We chose the constituent material of the system to minimize the adsorption or reaction with test gases. Given that the Teflon® polytetrafluoroethylene material was inert to most volatile chemicals, including 1,3-BD,¹⁴ we used 1.27-cm (0.5-in) internal diameter (ID) Teflon® tubing (Berghof/America [Concord, California]) to connect solenoid valves and the breathing mask. Teflon® sealer tape (Cole-Palmer Instrument Company [Vernon Hills, Illinois]) sealed the connection points, thus minimizing gas leakage.

Timing control of the experiment. Timing of the inhalation experiment (i.e., beginning, ending exposure, and collecting timed breath samples) was controlled by a computer program, which activated motor-driven solenoid valves (Honeywell 7000 Series pilot-operated valves, Honeywell, Inc. [New Britain, Connecticut]) in accordance with a predetermined time schedule. Solenoid

valves were controlled with an analog-to-digital signal converter (Model PPIO-08, Cyber Research, Inc. [New Haven, Connecticut]). The application electric voltage was 12-V direct current.

The user interface, written in Microsoft Visual Basic-4.0 by P. Y. Wang, was designed so that there was a clear graphic indication of the status of exposure test (Fig. 2). This design allows the operator to easily anticipate each sample, and the breath sampling bags can, therefore, be changed. The upper left center of the illustration (Fig. 2) shows the valve status (i.e., phase), indicating the air flow direction and scheduled time events in the inhalation test (i.e., event number). Each phase represents a set of valve positions, and each event represents the timing and duration of a phase in an inhalation test. "Begin" and "End" specify the timing of the beginning and end of the phases, respectively. For example, phase 3 represents exposure without sample collection; only 1 solenoid valve (DO_1) is turned

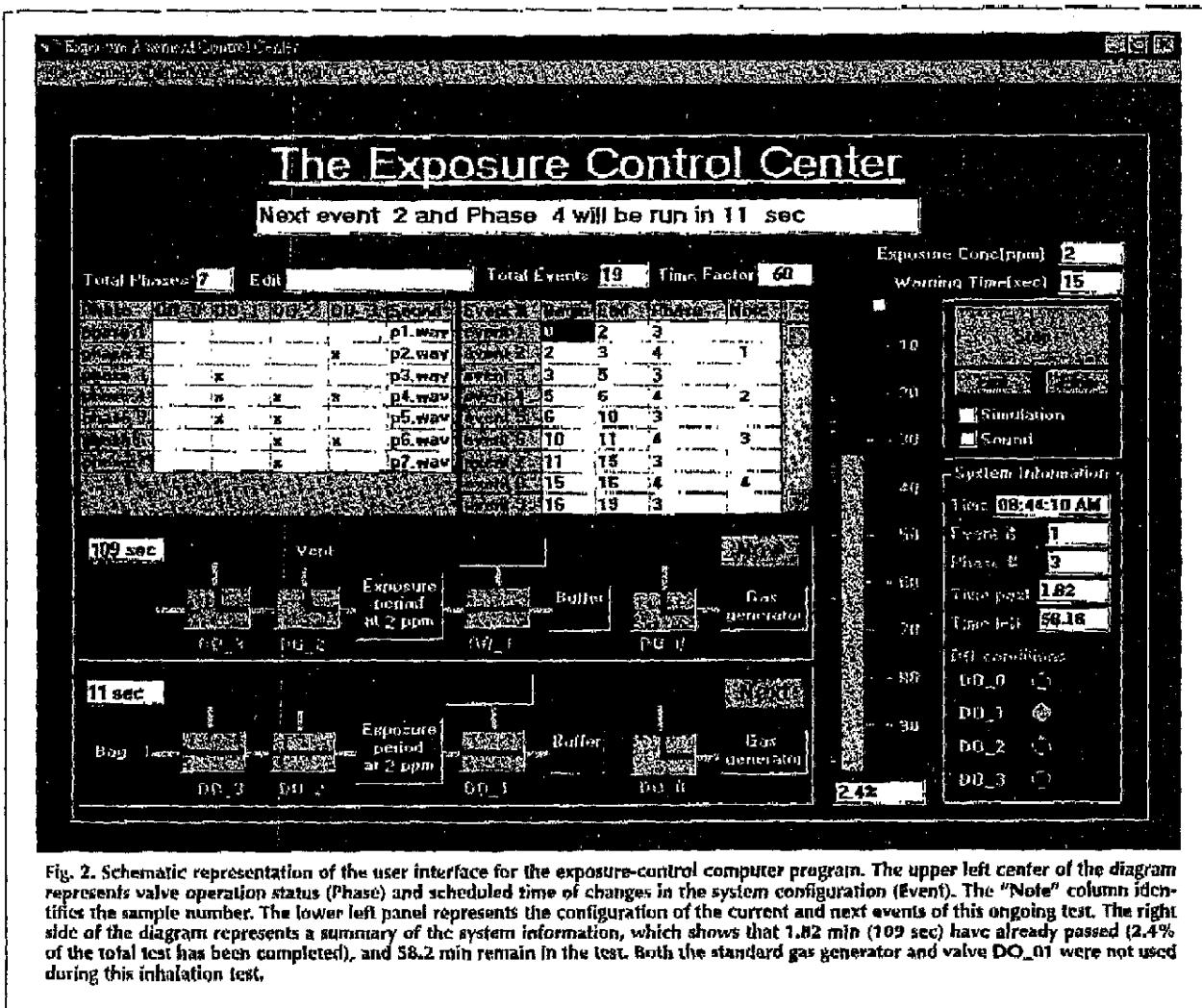


Fig. 2. Schematic representation of the user interface for the exposure-control computer program. The upper left center of the diagram represents valve operation status (Phase) and scheduled time of changes in the system configuration (Event). The "Note" column identifies the sample number. The lower left panel represents the configuration of the current and next events of this ongoing test. The right side of the diagram represents a summary of the system information, which shows that 1.82 min (109 sec) have already passed (2.4% of the total test has been completed), and 58.2 min remain in the test. Both the standard gas generator and valve DO_01 were not used during this inhalation test.

on (i.e., "x" in the D0_1 column), thus directing the gas flow from the buffer bag into the inhalation mask, and the other valves (i.e., D0_0, D0_2, and D0_3) are in the bypass position. In contrast, phase 4 represents exposure with sample collection. A desired exposure and sampling program can be constructed by assembling phases into a series of events. In this example (Fig. 2, central section), phases 3 and 4 are alternated, and periods of exposure are produced with and without breath sampling, respectively.

The rectangles in the lower left of the interface diagram represent the valve status for the current event and the next configuration of the coming event, respectively. The right portion of the diagram shows a summary of the system information: 1.82 min (109 sec) have already passed following the beginning of the test (i.e., 2.4% of the test has been completed), and 58.18 min remain in this inhalation test, which is shown visually by the black vertical bar. The standard gas generator was used only to provide purified air to prepare 2.0 ppm of 1,3-BD; both the standard gas generator and the first solenoid valve (D0_01) were shut down during the test.

Respiratory monitoring. Respiratory monitoring was performed with the RespiTrace breathing monitor with the RespiEvents data processing software (Nims, Inc. [Miami Beach, Florida]). This system estimates lung volume (total thoracic volume) of subjects by measuring the changing capacitance of wires embedded in elastic bands placed around the subject's chest and abdomen. The real-time respiratory kinetic information, calculated on the basis of lung volume, included breath-by-breath tidal volume, breathing frequency, and pulmonary minute ventilation.

Breath sample collection. The subject breathed through a face mask (Hans Rudolph, Inc. [Kansas City, Missouri]) that has 2, 1-way valves that can draw air in from the gas supply and can direct the exhalation to sampling bags. Each volunteer chose an optimal mask size (i.e., small, medium, or large). A mask leak test was also performed; the subject placed the palms of his or her hands over the mask's valves and attempted to inhale for 10 sec in an effort to detect any leakage. Exhaled air was collected in a Tedlar™ sampling bag and concentrated onto charcoal tubes treated with 4-tert-butylcatechol (4-tert-TBC) (SKC 226-73, 100/50 mg, SKC, Inc. [Eighty Four, Pennsylvania]). Treatment was achieved with a vacuum pump at a flow rate of 60 ml/min (224-44XR SKC personal air-sampling pump or flow-controlled vacuum). We used 4-tert-TBC to prevent self-polymerization of 1,3-BD on the charcoal tubes. Charcoal tubes were stored in a refrigerator at -20 °C until analysis. The mass of 1,3-BD and the volume of exhaled breath collected in each sample determined the exhaled 1,3-BD concentration.

Analysis of 1,3-BD. Analysis of 1,3-BD and the quality-control program were carried out in accordance with the modified analytical method of the U.S. National Institute for Occupational Safety and Health (NIOSH method 1024).¹⁵ A Hewlett Packard gas chromatograph/flame ionization detector 5890 series I and HP model 6890 autosampler were used for the sample

analysis. The analytical columns included a 50-m × 0.32-mm ID fused-silica, porous-layer (PLOT) column, coated with aluminum oxide/potassium chloride (Cat. No. 7515, Chrompack); and a back-flushable precolumn of 10 m × 0.5 mm ID, fused silica CP wax 57 CB (Cat. No. 7648, Chrompack). The initial oven temperature was held at 50 °C for 2 min, and it was then increased at a rate of 10 °C/min up to 200 °C, at which time it was held at 200 °C for 1 min. The purge time to begin the back-flush process was set at 0.6 min following injection. The charcoal was desorbed with 1.6 ml of methylene chloride (99.9%, Burdick & Jackson, Inc. [Muskegon, Michigan]), and the injection volume was 2 µl. The 6-point calibration curves over the range of 0.027–36.8 µg/ml were established with a stock solution of 148 µg/ml, which was prepared daily.

Overview of validation and evaluation. Disadvantages and measurement problems commonly found in breath-sampling techniques, including gas adsorption or gas leakage in the device, were investigated. For example, 1,3-BD is a reactive and unstable gas, and there was concern about 1,3-BD losses. The loss of 1,3-BD could occur at 3 points: (1) during chemical analysis, at which time loss could occur mainly from incomplete extraction of 1,3-BD from charcoal tubes; (2) during breath collection with Tedlar™ sampling bags; and (3) within the tubing and valves of the exposure system. We conducted validation tests to evaluate whether the performance of this inhalation system met the 3 aforementioned design goals; 8 duplicated trials were conducted for each validation test described below. Such an evaluation is required for any test gas used in the system.

Determination of 1,3-BD recoveries from the sampling media. We used 3 1,3-BD levels (i.e., 0.08 ppm, 0.4 ppm, and 2.0 ppm) to evaluate 1,3-BD losses during chemical analysis and breath sampling. We chose these levels on the basis of breath levels observed in pilot tests. The quantities of 1,3-BD, based on a breath sample of 5.0 l, were spiked directly into the charcoal tubes, thus enabling us to determine the recoveries in accordance with NIOSH 1024 method. We prepared Tedlar™ sampling bags by adding 1,3-BD into 5.0 l of purified air to simulate an exhaled breath sample: 0.88 µg, 4.42 µg, and 22.10 µg of 1,3-BD were used for concentration levels at 0.08 ppm, 0.4 ppm, and 2.0 ppm, respectively. The test gas was treated like a breath sample, and it was then collected onto charcoal tubes for chemical analysis.

Measurements of 1,3-BD with the exposure system. We used an exposure system designed for human inhalation study at a known concentration of test gas, followed by purified air (charcoal filtered) in the postexposure period. Losses of 1,3-BD in the inhalation system, including adsorption onto or reaction with the internal parts of the system, might affect the accuracy of the measurements of 1,3-BD. In addition, a carry-over problem might occur when the residual 1,3-BD left from exposure in the deadspace of the system (including connection tubing, valves, and the breathing mask) was washed out by purified air to the postexposure period. This carry-over problem might also affect the accuracy of 1,3-BD measurements, and it is most critical at the early postex-

posure phase (i.e., when exhaled 1,3-BD concentrations declined rapidly). We evaluated the device by simulating the collection of exhaled air in which 2.0 ppm of 1,3-BD was run through the system for 1 min, followed by purified air for another 2 min at a flow rate of 5.0 l/min. We collected simulated exhaled breath samples during and after exposure to estimate 1,3-BD losses and carry-over in the inhalation system, respectively. The device was tested for 1,3-BD losses with 2.0 ppm of 1,3-BD, inasmuch as this was the target exposure concentration at which human subjects were to be exposed in a companion study. Furthermore, if there were any losses of 1,3-BD through leakage, adsorption onto any part of this system, or carry-over of 1,3-BD, it would be most apparent and most likely detectable at 2.0 ppm, rather than at 0.4 ppm or 0.08 ppm of 1,3-BD. The criteria for acceptable errors in 1,3-BD measurements and carry-over were less than 10%, relative to reference standards, following correction for the recovery losses of 1,3-BD from charcoal tubes and Tedlar™ bags.

Evaluation of timing control. Precise timing control of the exposure duration and collection of exhaled breath are critical for the obtainment of valid data for pharmacokinetic studies. Changes in the inhaled gas source (i.e., 1,3-BD or purified air) and breath collection were done automatically with several 3-way solenoid valves in accordance with a prescheduled computer program (Fig. 2). To evaluate the precision in the timing control of the experiment, we used a digital stopwatch (Control Company [Friendswood, Texas]) with the resolution of 1/100th of a sec to evaluate the response time in the switching of the 3-way valves. The criteria for acceptable errors in timing control were less than ± 1 sec.

Validation of respiratory monitoring. Respiratory data were obtained using the RespTrace respiratory monitoring system. In addition to its internal 5-min qualitative diagnostic calibration (QDC), volumetric calibration of RespTrace with an external standard—an 8-l spirometer (Warren E. Collins [Braintree, Massachusetts])—was also done before and after each inhalation test. During calibration, the spirometer was placed in the position of the sample bag, and the subject's exhaled breath volume was measured for a series of 8 to 10 breaths. Furthermore, we compared measurements of minute ventilation with RespTrace with the volume of breath samples collected simultaneously (1-min duration).

Assessment of gas leakage. We used the leak test to determine if the exhaled breath was being collected completely. A general-pressure pump (Gast Manufacturing, Inc. [Benton Harbor, Michigan]), operated at a flow rate of 5.0 l/min, was used to evaluate the system. If the average difference between the input and output flow was more than 5%, it was defined as gas leakage. Flow measurement was performed with a DryCal DC-1SC primary volumetric flow meter (Bios International [Pompton Plains, New Jersey]). The instrument could detect the flow difference as low as 2% over the range from 0.01 l/min to 10 l/min.

Statistical analysis. We used linear regression analyses to establish the calibration curves for 1,3-BD standards vs. gas chromatography signal output (in a logarithmic

scale). The squared correlation coefficient (R^2) of the calibration curve was used as a quality control indicator for chemical analysis. Descriptive statistics, including the average recoveries and t tests, were used for the evaluation of the performance of the proposed inhalation system. The coefficient of variation (CV) (i.e., standard deviation divided by the mean, expressed in percentage) was used as an indicator of precision (repeatability) of measurements. The level of significance was set at .05.

Results

The squared correlation coefficient (R^2) of daily calibration curves ranged from 0.992 to 0.999 (in logarithmic scale), with an average pooled CV of 4.5% estimated from 3 replicates at each standard level. The reliable limit of quantification of gas chromatography analysis for 1,3-BD was estimated at 0.045 µg/ml, which corresponded to 0.006 ppm of 1,3-BD in a 5-l exhaled breath sample. The average recoveries from the charcoal tubes declined with decreasing 1,3-BD levels: 92.1% at 22.10 µg, 87.9% at 4.42 µg, and 71.4% at 0.88 µg (Table 1). All of the recoveries were significantly less than 100% ($p < .05$). These findings were comparable to the results of NIOSH method 1024. For the Tedlar™ sampling bags, the recoveries ranged from 79.2% to 85.4%, without correction for 1,3-BD recoveries from charcoal tubes. Following adjustment for 1,3-BD loss in the charcoal tube (i.e., division of the total recovered by the fraction retained by the charcoal tube), the average recoveries of 1,3-BD from Tedlar™ bags ranged from 92.7% to 110.9%. It appeared that the loss of 1,3-BD was much less in the Tedlar™ bags than in the charcoal tubes. Overall, there was an average recovery of 99.1%, or a 0.9% loss from the bags, although it averaged 93.1% for 2.0 ppm and 0.4 ppm—excluding the losses from the charcoal. This result implies that only a small amount of 1,3-BD (perhaps as much as 6.9%) was lost during the collection and transfer of breath samples to the charcoal tubes.

Following correction for recovery losses from collection and analytical procedures, 1,3-BD loss in the proposed system was negligible—an error of 2.1% relative to the reference standard of 2 ppm 1,3-BD (Table 2). Carry-over of 1,3-BD into the postexposure period was most apparent during the first minute after the valves were switched from 1,3-BD to clean air. During this time period, the average carry-over (0.049 ± 0.006 ppm [mean \pm standard deviation]) was less than 2.5% of the previous 1,3-BD exposure, and it declined rapidly for later time periods; only 1.1% was found in the second minute following cessation of exposure. The overall precision in 1,3-BD measurements was 9.8%. There was no evidence of gas leakage in the system, and timing control of the valve operation was precise. For a 5.0 l/min input, the average output reading was 4.9 ± 0.4 l/min. The difference between input and output flow was not statistically significant ($p = .3$). The measured response time in the switching of valves was 0.13 ± 0.02 sec, which was within the validation criteria of ± 1.0 sec.

Figure 3 shows an example of time curves of alveolar

Table 1.—Recoveries of 1,3-Butadiene (1,3-BD) from a Charcoal Tube and a Tedlar™ Bag*

Spiked 1,3-BD (µg)	Charcoal tube				Tedlar™ bag				Corrected 1,3-BD recovery (%)\$	
	Recovered 1,3-BD (µg)		1,3-BD recovery (%)†		Recovered 1,3-BD (µg)		1,3-BD recovery (%)‡		x	SD
	x	SD	x	SD	x	SD	x	SD		
0.88	0.63	0.06	71.4	6.8	0.70	0.09	79.2	9.1	110.9	14.3
4.42	3.89	0.15	87.9	3.4	3.64	0.31	82.3	7.0	93.6	8.0
22.1	20.35	0.57	92.1	2.6	18.87	1.30	85.4	5.9	92.7	6.4

Notes: x = mean, and SD = standard deviation.

*Eight replicate tests were conducted at each 1,3-BD level for both the charcoal tube and Tedlar™ bag.

†Equal to 1,3-BD recovered from the charcoal tube × 100%/spiked 1,3-BD.

‡Equal to 1,3-BD recovered from the Tedlar™ bag × 100%/spiked 1,3-BD, uncorrected for the recovery of 1,3-BD from the charcoal tube.

\$Equal to 1,3-BD recovered from the Tedlar™ bag × 100%/spiked 1,3-BD, corrected for the recovery of 1,3-BD from the charcoal tube.

Table 2.—Evaluation of 1,3-Butadiene (1,3-BD) Measurements and Carry Over with the Proposed System*

Time interval of collection (min)	Time interval characteristics	Expected 1,3-BD concentration (ppm)	Measured 1,3-BD concentration (ppm)†	
			x	SD
0–1	Exposure (wash-in)	2.0	2.04	0.19
1–2	Postexposure (wash-out)	0.0	0.049	0.006
2–3	Postexposure (wash-out)	0.0	0.021	0.003

Notes: x = mean, and SD = standard deviation.

*Eight replicate tests were conducted.

†Corrected for 1,3-BD recoveries of charcoal tube and Tedlar™ sampling bag.

1,3-BD concentration obtained with this system during tests on 3 volunteers. These volunteers were tested as part of Mezzetti's study (in preparation) for the development of a physiologically based pharmacokinetic model, with parameters fitted for each subject. The experiments were approved by the Institutional Review Board at the Harvard School of Public Health under the Department of Health and Human Services regulations (identification number M1208, IRB number 01). The demographic characteristics of these 3 subjects are summarized in Table 3. Each subject was exposed to 2.0 ppm of 1,3-BD for 20 min, followed by purified clean air for an additional 40 min. Ten timed exhaled breath samples were collected and analyzed for 1,3-BD in accordance with a predetermined schedule. The first 5 timed exhaled breath samples were collected at 2 min, 5 min, 10 min, 15 min, and 19 min during 1,3-BD exposure. The other 5 breath samples were collected at 21 min, 22 min, 28 min, 38 min, and 58 min following the initiation of exposure while the subject inhaled purified air. Each of the first 7 breath samples was collected for 1 min, and the final 3 samples were collected for 2 min

each, thus increasing the sensitivity for detection of exhaled 1,3-BD. The subject provided a baseline breath sample to verify that there were no background 1,3-BD sources that could interfere with the experiment. The difference between the pulmonary minute ventilation for which RespiTrace was used and the directly measured volume of exhaled breath in the bag collected simultaneously was less than 10%. Potential gas leakage around the face mask was minimized by (1) provision of the optimal mask size for each subject and (2) requesting that the subject avoid unnecessary movement during the test. Obviously, small differences in the timing of sample collections could affect the shape of the curve, especially at the change over from exposure to clean air. The reproducibility of exposures and breath sampling across the test population is critical for the obtainment of comparable data.

Discussion

To our best knowledge, the proposed system herein is the first computer-based, face-mask exposure device to

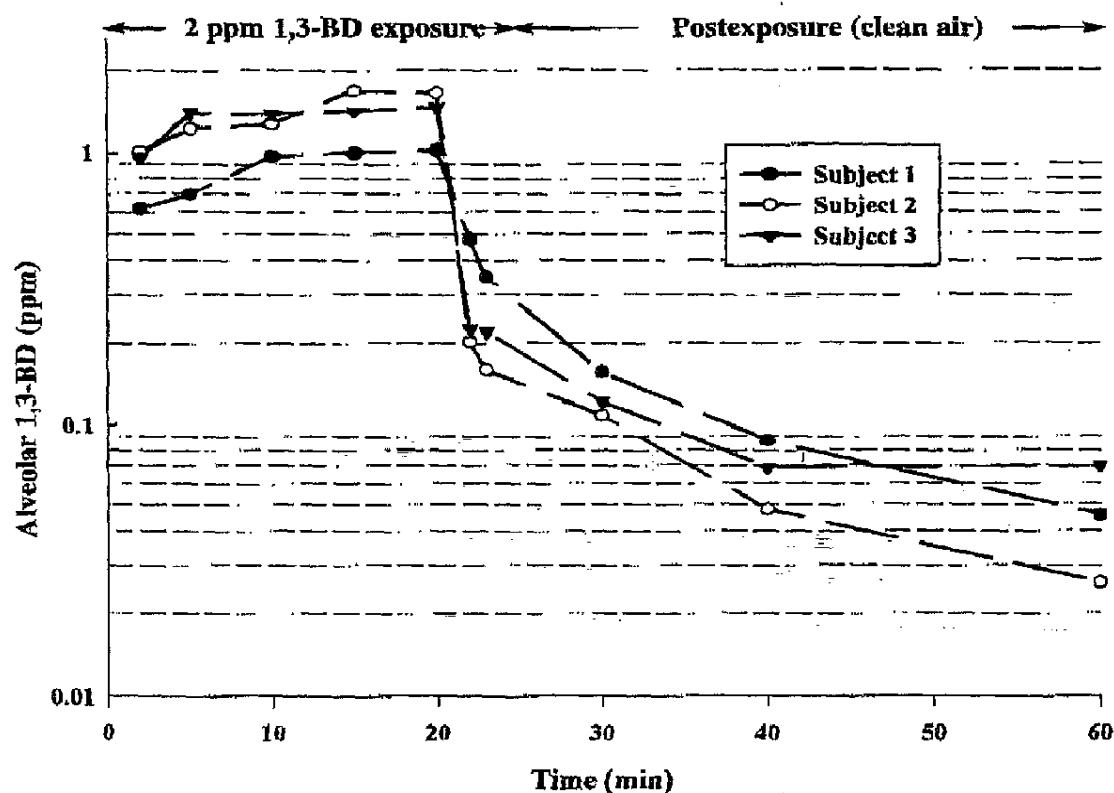


Fig. 3. Time course of mean alveolar 1,3-butadiene (1,3-BD) during exposure and postexposure phases summarized from 3 subjects exposed to 2 ppm 1,3-BD for 20 min, followed by clean air for an additional 40 min.

be used for a large-scale human-inhalation study; tests for which this system has been used have been conducted on more than 130 individuals. The device minimizes problems with the timing of breath sampling and with carry-over after exposure. This feature is critical because the initial phase of the washout curve for an inhaled gas has important information about the internal transport and metabolism of the gas that can be captured with pharmacokinetic modeling. Inasmuch as breath concentrations decline rapidly during the initial phase of washout (Fig. 3), it is important that the carry-over effects be minimized and that there is precise timing in breath sampling at the early phase of the washout period. On the basis of the validation results, both timing control errors and carry-over of the test gas were negligible in this system.

All of the exhaled breath, collected for a defined period of time, is a sample of mixed exhaled breath that contains both unexchanged air from the deadspace in the airways and exchanged air from the alveoli. This mixture provides more material for analysis than a single end-tidal sample of alveolar air from 1 breath, and it lowers the limit of detection. However, the mixed exhaled breath does not truly represent the concentration

of the test gas in alveolar air, given that the deadspace does not contribute to gas exchange with pulmonary blood, and it remains largely unchanged. We can estimate the alveolar gas concentration by adjusting mixed exhaled breath for the contribution from the deadspace. A good estimate ($R^2 = .89$) of the deadspace was obtained, given a subject's age, height, sex, breathing frequency, tidal volume, and use of the algorithm developed by Harris et al.¹⁶ However, if the deadspace is to be estimated effectively, each subject's breathing pattern must be monitored carefully, thus providing valid measurements of tidal volume and breathing frequency. In contrast, existing methods for directly measuring alveolar gas are either quite invasive and complicated (e.g., tracheal intubation) or there is poor sensitivity because only a limited volume is available for analysis (e.g., collection of the end of a single exhalation¹⁷).

Compared with conventional chamber approaches for exposure and manual breath sampling, this system is reliable and represents easy-to-use technology. Problems have been mostly limited to fitting and use of the face mask. One subject could not tolerate breathing through a face mask, and a few volunteers could not obtain an adequate fit with the available masks. The